



MINI-REVIEW

Mineralization/Anti-Mineralization Networks in the Skin and Vascular Connective Tissues

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CME Accreditation Statement: This activity ("ASIP 2013 AJP CME Program in Pathogenesis") has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of the American Society for Clinical Pathology (ASCP) and the American Society for Investigative Pathology (ASIP). ASCP is accredited by the ACCME to provide continuing medical education for physicians.

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CME Disclosures: The authors of this article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.

Accepted for publication
March 12, 2013.

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Ectopic mineralization has been linked to several common clinical conditions with considerable morbidity and mortality. The mineralization processes, both metastatic and dystrophic, affect the skin and vascular connective tissues. There are several contributing metabolic and environmental factors that make uncovering of the precise pathomechanisms of these acquired disorders exceedingly difficult. Several relatively rare heritable disorders share phenotypic manifestations similar to those in common conditions, and, consequently, they serve as genetically controlled model systems to study the details of the mineralization process in peripheral tissues. This overview will highlight diseases with mineral deposition in the skin and vascular connective tissues, as exemplified by familial tumoral calcinosis, pseudoxanthoma elasticum, generalized arterial calcification of infancy, and arterial calcification due to CD73 deficiency. These diseases, and their corresponding mouse models, provide insight into the pathomechanisms of soft tissue mineralization and point to the existence of intricate mineralization/anti-mineralization networks in these tissues. This information is critical for understanding the pathomechanistic details of different mineralization disorders, and it has provided the perspective to develop pharmacological approaches to counteract the consequences of ectopic mineralization. (*Am J Pathol* 2013, 183: 10–18; <http://dx.doi.org/10.1016/j.ajpath.2013.03.002>)

Ectopic Mineralization: A Global Pathological Problem

Ectopic mineralization (ie, deposition of calcium/phosphate complexes in connective tissues in aberrant locations) has been linked to several clinical conditions, such as aging, cancer, diabetes, and autoimmune diseases, major causes of morbidity and mortality. For example, a recent study has examined the risk of death associated with coronary artery calcification in a cohort of 25,253 patients and found that coronary artery calcification is an independent risk factor to death, with the relative risk being up to 12.5-fold.¹

Two major types of acquired ectopic mineralization processes involving peripheral connective tissues have been

recognized. Metastatic calcification refers to calcium deposition associated with elevated serum levels of phosphate and/or calcium, as in chronic renal failure, whereas dystrophic calcification is usually secondary to some form of insult to the tissues, as seen in autoimmune diseases and cancer. Metastatic calcinosis in the skin can be characterized clinically by nodular deposits of calcium and phosphate, often in peri-articular distribution, and they are reversible on correction of the calcium and/or phosphate abnormalities.² This can also be seen in association with vascular calcification, as in calciophylaxis, which is associated with an approximately 70%

Supported by NIH/National Institute of Arthritis and Musculoskeletal and Skin Diseases grants R01 AR28450 and R01 AR55225 (J.U.) and a Dermatology Foundation Research Career Development Award (Q.L.).

mortality rate, and it commonly occurs in patients with chronic renal failure, resulting in hyperphosphatemia.³

Dystrophic calcification occurs frequently in previously damaged or diseased tissues. Localized skin involvement occurs in many cutaneous inflammatory lesions, such as acne, chronic ulcers, and granulomas, as well as in benign and malignant neoplasms.² Although calcinosis cutis can present as small cutaneous deposits primarily on the fingers or elbows in patients with systemic sclerosis, it has also been reported in patients with systemic lupus erythematosus and dermatomyositis.^{4,5} In these conditions, dystrophic calcification occurs without serum calcium/phosphate abnormalities. Thus, ectopic mineralization represents the consequence of several contributing metabolic and environmental factors, making the uncovering of the precise basis of these disorders in the populations at large exceedingly difficult.

Heritable Diseases of Ectopic Mineralization

Skin Diseases with Mineral Deposition: Familial Tumoral Calcinosis

Several Mendelian genetic disorders share phenotypic similarities with the acquired forms of metastatic and dystrophic calcification, and serve as genetically controlled model systems to study various facets of pathological mineralization (Table 1). Recent studies on these conditions, with accompanying animal models, have allowed identification of several genetic factors that contribute to ectopic mineralization, providing evidence of intricate mineralization/anti-mineralization networks in peripheral connective tissues (Figure 1).

Two forms of familial tumoral calcinosis affect primarily skin and recapitulate features of acquired forms of metastatic and dystrophic calcification. The hereditary counterpart of acquired metastatic calcinosis is hyperphosphatemic familial tumoral calcinosis (HFTC; Mendelian Inheritance of Man 211900), an autosomal recessive condition characterized by progressive deposition of calcium phosphate crystals in periarticular spaces and soft connective tissues.^{6,7} As in acquired cases of metastatic calcification, HFTC is associated with marked hyperphosphatemia. Mutations in one of three genes involved in the regulation of phosphate excretion in the kidney may result in the HFTC phenotype (Figure 1). First, loss-of-function mutations in the fibroblast growth factor 23 gene (*FGF23*) were shown to cause HFTC.⁸ The protein encoded by *FGF23* controls phosphate re-absorption in the kidney, and when *FGF23* is not functioning properly, phosphate excretion is impaired and high levels of phosphate accumulate in the circulation. Second, HFTC-causing mutations were identified in *GALNT3*,⁹ a gene encoding UDP-*N*-acetyl- α -D-galactosamine:polypeptide *N*-acetylgalactosaminyl-transferase 3, a glycosyltransferase that initiates mucin-type O-glycosylation. Mechanistically, O-glycosylation of FGF23 by this enzyme prevents proteolytic cleavage and inactivation of FGF23, thus allowing its action under physiological conditions.^{10,11} Finally, mutations in the *KLOTHO* gene, encoding a coreceptor for FGF23, were found to be associated with HFTC.^{12,13} Hence, the study of HFTC, a rare genetic disorder, has led to the understanding of complex biochemical processes of relevance to common acquired diseases.

Table 1 Human Diseases with Ectopic Mineralization Phenotypes, Genes, and Corresponding Mutant Mouse Models

Human disease	Phenotypic features	Gene, protein	Mouse model
PXE	Mineralization in the skin, eyes, and cardiovascular system	<i>ABCC6</i> , ATP-binding cassette C subfamily, member 6	<i>Abcc6</i> ^{-/-} ; KK/HlJ
GACI	Arterial calcification and joint and spine ossification	<i>ENPP1</i> , nucleotide pyrophosphatase	<i>Enpp1</i> ^{-/-} ; <i>ttw/ttw</i> ; <i>asj</i>
ACDC	Vascular and joint calcification	<i>NT5E</i> , CD73	<i>Nt5e</i> ^{-/-}
NFTC	Ulcerative mineralization lesions in skin	<i>SAMD9</i> , sterile α motif domain containing 9	
HFTC	Mineralization masses in skin	<i>KL</i> , klotho; <i>GALNT3</i> , ppGaNase-T3; <i>FGF23</i> , fibroblast growth factor 23	<i>Klotho</i> ^{-/-} ; <i>Galnt3</i> ^{-/-} ; <i>Fgf23</i> ^{-/-}
Multiple vitamin K-dependent coagulation factor deficiency	Vitamin K-dependent coagulation factor deficiency and PXE-like skin changes	<i>GGCX</i> , vitamin K-dependent γ -carboxylase	<i>Ggcx</i> ^{-/-}
AI	Arterial, pulmonary, and eye calcification	<i>FAM20A</i> , family with sequence similarity 20, member A	<i>Fam20a</i> ^{-/-}
Keutel syndrome	Arterial and cartilage calcification	<i>MGP</i> , matrix gla protein	<i>Mgp</i> ^{-/-}
Familial IBGC	Vascular and pericapillary calcification in brain	<i>SLC20A2</i> , sodium-dependent phosphate transporter 2	
FHH	Cataracts, vibrissae, muscle, and vascular calcification	<i>CASR</i> , calcium-sensing receptor	<i>Casr</i> ^{-/-}

ACDC, arterial calcification due to CD73 deficiency; AI, amelogenesis imperfecta; GACI, generalized arterial calcification of infancy; HFTC, hyperphosphatemic familial tumoral calcinosis; IBGC, idiopathic basal ganglia calcification; NFTC, normophosphatemic familial tumoral calcinosis; PXE, pseudoxanthoma elasticum.

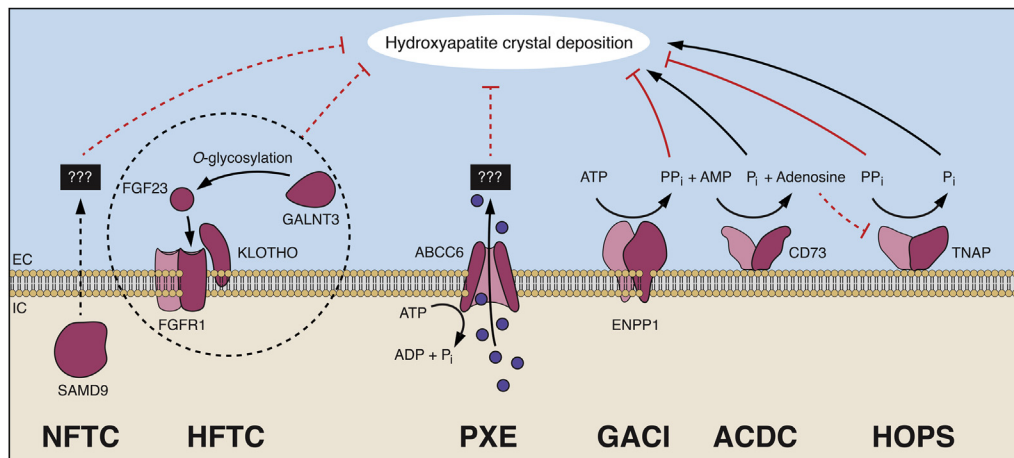


Figure 1 Genetic complexity of the mineralization/anti-mineralization network. Mutations in specific genes (also in Table 1) can result in deposition of hydroxyapatite in several heritable ectopic mineralization disorders. The **blue circles** represent unidentified anti-mineralization factors transported by ABCC6 under physiological conditions from the intracellular milieu (IC) to the extracellular space (EC). HOPS, infantile hypophosphatasia.

The second type of familial tumoral calcinosis, normophosphatemic familial tumoral calcinosis (NFTC; Mendelian Inheritance of Man 610455), recapitulates features of acquired dystrophic calcinosis and manifests with extensive ectopic mineralization of the cutaneous tissues, associated with preceding inflammatory manifestations mostly evident in mucosal tissues. NFTC results from mutations in the *SAMD9* gene,^{14,15} which encodes an approximately 170-kDa protein with unknown function. Gene expression profiling of cell cultures derived from an aggressive fibromatosis tumor with an adenomatosis polyposis coli mutation has shown *SAMD9* to be up-regulated on transfection with the wild-type adenomatosis polyposis coli, although *SAMD9* expression was lower in aggressive fibromatosis.¹⁶ Also, reduced levels of *SAMD9* expression were noted in 20% of cases of breast cancer and 35% of cases of colon cancer cell lines compared with normal tissue. Moreover, cancer cells transfected with *SAMD9* expression vector that were injected into mice produced tumors of lower volume compared with cancer cells transfected with an empty vector. Thus, *SAMD9* may play a role as a tumor-suppressor gene, but the relevance of these observations to the mineralization phenotype remains unclear.¹⁶ Nevertheless, given that *SAMD9* deficiency in NFTC causes clinical manifestations of inflammation and because *SAMD9* has been shown to regulate apoptosis, it has been speculated that tumor necrosis factor (TNF)- α , a cytokine that links inflammation and apoptosis,¹⁷ might have a role in *SAMD9* regulation. Indeed, treatment of cells with TNF- α caused a sixfold increase in *SAMD9* transcription, and the increase in *SAMD9* expression was mediated through a p38 proinflammatory and proapoptotic pathway.¹⁵ In addition, inhibition of NF- κ B, a factor that antagonizes the proapoptotic response, caused a considerable increase in *SAMD9* expression after treatment with TNF- α , thus suggesting that *SAMD9* functions, along the TNF- α signaling pathway, as a possible repressor of this system.

Multisystem Mineralization Disorders: Pseudoxanthoma Elasticum

A prototype of multisystem ectopic mineralization disorders is pseudoxanthoma elasticum (PXE), characterized by protean manifestations in the skin, the arterial blood vessels, and the eyes.¹⁸ PXE is inherited in an autosomal recessive manner and is caused by mutations in the *ABCC6* gene that encodes ABCC6, a transmembrane efflux transporter. Interestingly, ABCC6 is expressed primarily in the liver, to some extent in the kidneys and the intestine, but at low levels, if at all, in tissues directly affected by mineralization in PXE.¹⁹ Clinical observations on PXE, including late onset of manifestations and slow, yet progressive, development of the phenotype, coupled with studies using a knockout mouse model, *Abcc6*^{-/-}, as a platform (see later), have suggested that PXE is a metabolic disorder with a primary molecular defect in the liver.²⁰ However, the precise pathomechanistic details leading from *ABCC6* mutations to mineralization in peripheral tissues are unknown.²¹

The clinical manifestations of PXE are usually noted first in the skin, which manifests with small yellowish papules on the predilection sites, such as the side of the neck and the antecubital fossae.²² Although the cutaneous findings by themselves are only of cosmetic concern, they signify the potential for development of serious eye problems. Characteristic eye lesions consist of angioid streaks that result from breakage in the mineralized Bruch's membrane behind the retina. The angioid streaks are accompanied by capillary breakage, bleeding to the eye, and neovascularization resulting in progressive loss of visual acuity and central blindness in most patients, if left untreated.²³ The involvement of the cardiovascular system reflects mineralization of medium-sized arterial blood vessels and clinically manifests with hypertension, intermittent claudication, occasional bleeding from the intestinal arteries, and, rarely, early myocardial infarcts and stroke.²⁴

PXE is a rare disease, with an estimated prevalence of approximately 1:50,000, which implies that there are approximately 7000 affected individuals in the United States and as many as 150,000 in the world, assuming the same global prevalence.²⁵ Most patients have mutations in the *ABCC6* gene, and approximately 600 distinct loss-of-function mutations have been encountered, including recurrent p.R1141X and g.del23-29, which account for up to approximately 45% of all mutations.²⁶ Recent clinical observations of the presence of PXE-like cutaneous findings associated with unusual phenotypes have revealed additional mutated genes. One of them is vitamin K–dependent coagulation factor deficiency, manifesting primarily with a bleeding disorder. In addition, some of these families develop unusually loose and lax skin with features of PXE.^{27,28} These patients harbor mutations in the *GGCX* gene, which encodes vitamin K–dependent γ -glutamyl carboxylase. This enzyme catalyzes γ -carboxylation of glutamyl residues in several proteins, so-called Gla proteins, which include several coagulation factors and matrix Gla protein (MGP).²⁹ MGP is present in peripheral connective tissues serving as a powerful anti-mineralization factor when in fully carboxylated form. Thus, as a result of inactivating mutations in the *GGCX* gene, the MGP remains uncarboxylated, thus allowing ectopic mineralization of the peripheral connective tissues in the skin and arterial blood vessels to ensue. Based on these observations, it has been suggested that ectopic mineralization in PXE reflects deficiency of vitamin K, similarly leading to reduced levels of fully carboxylated and, hence, active MGP.³⁰ This hypothesis has been tested by feeding *Abcc6*^{−/−} mice with high doses of vitamin K1 or K2 or by intravenous injection of vitamin K–glutathione conjugate.^{31–33} None of these treatments resulted in amelioration or prevention of the ectopic mineralization in this mouse model of PXE.

The role of vitamin K has also been examined by feeding *Abcc6*^{−/−} mice with warfarin, an anticoagulant that interferes with the vitamin K cycle by preventing the reduction of oxidized vitamin K (epoxide) to its reduced form (hydroquinone).³⁴ Clinical use of warfarin has been reported to be associated with mineralization of the arterial blood vessels and cardiac valves.³⁵ Feeding *Abcc6*^{−/−} mice with a warfarin-containing diet resulted in massive accumulation of mineral deposits in multiple tissues, well beyond the levels noted in *Abcc6*^{−/−} mice fed a control diet. These results suggested that further reduction in the γ -glutamyl carboxylation of MGP, caused by warfarin feeding, resulted in severe ectopic mineralization. The clinical implication of these experiments is that patients with PXE may be at risk of worsening of their disease as a result of warfarin therapy.³⁴

Heritable Disorders of Vascular Mineralization: GACI and ACDC

Generalized arterial calcification of infancy (GACI), an autosomal recessive disorder, has provided information on the postulated mineralization/anti-mineralization networks in

the skin and vascular connective tissues.³⁶ GACI and PXE have been considered to be clinically two distinct conditions in their classic forms: vascular mineralization in PXE is of late onset, with accompanying skin and eye findings. However, patients with GACI are often diagnosed as having severe vascular mineralization prenatally or at birth, and most of the latter patients die within the first year of life. GACI is caused by mutations in the *ENPP1* gene, encoding nucleotide pyrophosphatase phosphodiesterase, an enzyme that hydrolyzes ATP to generate AMP and pyrophosphate (PP_i), a powerful anti-mineralization factor^{36,37} (Figure 1). Recently, however, several families with characteristic features of GACI have been shown to harbor mutations in the *ABCC6* gene, and surprisingly, many of these mutations have been previously shown to cause PXE in unrelated families.^{38,39} Of interest is a report on a family in which one of the siblings died in early infancy, with clinical findings consistent with GACI, whereas another sibling developed late-onset manifestations characteristic of PXE, shown to be associated with mutations in *ABCC6*.⁴⁰ Attesting to the clinical overlap of PXE and GACI is also a report on a 2-year-old patient with clinical findings of vascular calcification diagnosed as having GACI and with associated characteristic cutaneous findings of PXE.⁴¹ This patient was shown to harbor a homozygous p.Y513C mutation in *ENPP1*. Thus, mutations in the *ENPP1* and *ABCC6* genes can present with phenotypic similarities between GACI and PXE, attesting to the possibility of divergent mineralization pathways due to underlying mutations in distinct genes.⁴²

Another recently described clinical entity with vascular involvement with similarities to PXE is arterial calcification due to CD73 deficiency (ACDC) caused by mutations in *NT5E*.⁴³ The *NT5E* gene encodes CD73, an enzyme that is linked to plasma membrane by glycosylphosphatidylinositol and breaks down AMP to adenosine and inorganic phosphate (P_i) (Figure 1). Although the mineralization affects primarily arteries in the lower extremities, as in PXE, several histological differences have been indicated, suggesting different pathological processes.⁴⁴ As a consequence of CD73 deficiency, the activity of tissue non-specific alkaline phosphatase is increased, accelerating the conversion of PP_i to P_i, a pro-calcifying factor (Figure 1). Furthermore, despite clinical differences in the severity and localization of the vascular calcification between GACI and ACDC deficiency, they both reflect alterations in PP_i/P_i metabolism and adenosine signaling. How these observations relate to vascular mineralization in PXE remains to be explored. However, recent data have specifically indicated that *ABCC6* does not serve as an efflux pump for adenosine under physiological conditions.⁴⁵

Mouse Models of Heritable Ectopic Mineralization Disorders

Understanding of the heritable ectopic mineralization disorders has been advanced significantly by examination of animal models that often recapitulate the features of human

disease. In addition to providing information on the pathomechanistic pathways and modifying factors, many of these mouse models have served as a platform to explore treatment modalities for these often intractable disorders. An example of a disease in which significant progress has been made toward understanding the underlying pathomechanisms through study of mouse models is PXE. Specifically, the *Abcc6*^{-/-} mice, developed by targeted ablation of the corresponding mouse gene, recapitulate the genetic, histopathological, and ultrastructural features of the disease.^{46,47} An early puzzling observation on PXE related to the fact that the clinical manifestations, as a result of ectopic tissue mineralization, affect primarily the skin, the vascular connective tissues, and the eyes; however, the gene harboring mutations in this disorder, *ABCC6*, is expressed predominantly in the liver, and at a low level, if at all, in clinically affected tissues.¹⁹ This dilemma has been resolved by mouse studies that have demonstrated that PXE is a primary metabolic disorder, rather than a connective tissue disease, as suggested earlier, and the primary pathological characteristics reside in the liver, with subsequent downstream manifestations in peripheral connective tissues.²⁰ This conclusion was reached, in part, by parabiotic pairing of *Abcc6*^{-/-} mice with their wild-type littermates.⁴⁸ After having successfully established a shared circulation, it was demonstrated that knockout mice in *Abcc6*^{-/-}; wild-type pairing did not develop ectopic mineralization, in contrast to their counterparts in *Abcc6*^{-/-}; *Abcc6*^{-/-} pairings. This and related observations suggested that the mineralization phenotype in PXE is a result of lack of an anti-mineralization factor in the circulation, which, under normal physiological conditions, prevents calcium phosphate precipitation. It was then postulated that such a factor, under normal physiological conditions, is transported out of the liver to the circulation by *ABCC6*, and in the absence of functional transporter activity in PXE, such factor is missing in circulation, allowing ectopic tissue mineralization to ensue.¹⁸

Several factors have been suggested to be the critical molecule transported by *ABCC6*. The importance of identifying the missing anti-mineralization factor in circulation of patients with PXE is emphasized by the fact that once the identity of such a molecule has been disclosed, it can be introduced directly to the circulation bypassing the liver, and this approach would be expected to prevent further mineralization. Based on clinical observations (previously described), vitamin K or one of its derivatives has been suggested to be the molecule whose absence in peripheral tissues would lead to deficient γ -glutamyl carboxylation of MGP and accumulation of inactive MGP.³⁰ This suggestion was supported by observations that MGP in the skin of patients with PXE and in tissues of *Abcc6*^{-/-} mice is undercarboxylated.^{49,50} Furthermore, it has been suggested that the vitamin K levels in the serum of patients with PXE are somewhat reduced.⁵¹ However, feeding of *Abcc6*^{-/-} mice with massive quantities of vitamin K did not prevent or reverse the mineralization process.^{31–33} Furthermore,

subsequent liver perfusion experiments in *Abcc6*^{-/-} mice and in *Abcc6* vesicle transport systems failed to provide evidence that vitamin K would be transported by *ABCC6*.⁵²

In addition to *Abcc6*^{-/-} knockout mice, four naturally occurring inbred mouse strains have been recently shown to demonstrate connective tissue mineralization similar to *Abcc6*^{-/-} mice.^{53,54} Further examination of these mice revealed the presence of a single-nucleotide polymorphism in *Abcc6*, which is predicted to result in substitution of arginine by cysteine (p.R621C), but also to interfere with splicing of the *Abcc6* pre-mRNA. As a result, *Abcc6* protein levels in the liver were markedly reduced, indicating that these naturally occurring strains of mice are allelic to the *Abcc6*^{-/-}. However, the four inbred strains harboring the same *Abcc6* mutation displayed mineralizing phenotypes that were highly variable, attesting to the notion that the genetic background of mice, and presumably of patients with PXE, can modify the mineralization phenotype. Thus, these mouse models provide an opportunity to identify specific modifier genes by genome-wide association studies and quantitative trait locus analyses. In fact, the development and the severity of the mineralization phenotype in *Abcc6*^{-/-} mice has already been shown to be modulated by the *Ggcx* gene, and specifically, the mineralization phenotype of *Abcc6*^{-/-} mice was accelerated when the mice were placed on a *Ggcx*^{+/-} background, as compared with *Abcc6*^{-/-}; *Ggcx*^{+/+} mice.⁵⁵ This observation is in support of digenic inheritance previously shown to occur in a family with PXE-like cutaneous findings and with a heterozygous *ABCC6* mutation in one allele and a heterozygous *GGCX* mutation in the other allele.⁵⁶

In addition to *Abcc6*^{-/-} mice recapitulating PXE, several mouse models for other heritable mineralization disorders affecting the skin and/or the vascular connective tissues have been described, including mice with mutant *Enpp1* as a model for GACI (Table 1). Several mouse models have been shown to depict ectopic mineralization, some in the dermal sheath of vibrissae and others in arterial blood vessels, yet no human disease counterparts have been recognized as of yet with mutations in the homologous genes (Table 2). However, many of these conditions appear to provide clues to the pathomechanisms of ectopic mineralization.

Mechanisms of Soft Tissue Mineralization

The mineral deposits in connective tissues in patients with PXE and in *Abcc6*^{-/-} mice have been shown to consist of calcium and phosphate that colocalize within the histologically demonstrable lesions, as determined by energy-dispersive X-ray.^{22,57} The ratio of calcium/phosphate progressively increases with the maturation of the mineralized lesions, reaching a value of approximately 2.0, comparable to the endochondral bone.⁵⁷ The progressive increase in mineralization is also reflected by an increased mineral/matrix ratio, as determined by Fourier-transform infrared imaging spectroscopy, and determination of the mineral phases suggests progressive maturation of the mineral deposits from amorphous

Table 2 Mouse Models of Ectopic Mineralization without Corresponding Human Disease

Gene, protein	Mouse model	Phenotypic features
AHSG, α 2-HS-glycoprotein/fetuin-A	<i>Ahsg</i> ^{-/-}	Vascular calcification
ADIPOQ, adiponectin	<i>Apoe</i> ^{-/-}	Aortic calcification
ANK, progressive ankylosis	<i>Ank</i> ^{-/-}	Arterial and cartilage calcification
CA2, carbonic anhydrase 2	<i>Car2</i> ^{-/-}	Arterial calcification, osteopetrosis
OPN, osteopontin	<i>Opn</i> ^{-/-}	No mineralization defect per se, but enhanced vascular calcification in <i>Mgp</i> ^{-/-} mice
OPG, osteoprotegerin	<i>Opg</i> ^{-/-}	Osteoporosis, vascular calcification
SMAD6, SMAD family member 6	<i>Madh6</i> -mutant	Aortic ossification
TIF1A, transcriptional intermediary factor 1 α	<i>Tif1a</i> ^{-/-}	Calcification of arteries, vibrissae, and lungs

calcium phosphate to hydroxyapatite. This mineralization process may be affected by the availability of the nucleation sites in extracellular elastic structures and/or collagen fibers and by a tissue-specific microenvironment that favors mineralization, including the local concentrations of calcium and phosphate and the presence of local and systemic anti-mineralization factors. An explanation for the mineralization may relate to the matrix-vesicle (MV) nucleated mineralization, and specifically, MVs have been shown to play an important role in both skeletal and ectopic mineralization.⁵⁸ It has, indeed, been suggested that pathological mineralization of soft connective tissues may have mechanisms similar to skeletal calcification, and MVs may play a role in both mechanisms by serving as a nucleation site. Vascular calcification has also been suggested to follow the biomineralization pathway, in which MV or MV-like particles in the tissue modulate the local $\text{PO}_4^{3-}/\text{PP}_i$ ratio. Because PP_i is a powerful inhibitor of mineral formation, changing the P_i/PP_i ratio could either trigger or inhibit mineral formation, depending on the precise balance of these ions. Irrespective of the precise mechanisms, however, understanding of the overall mineralization processes and of the composition of the final deposits can provide information relevant to potential inhibition of the formation of early precursors and their transformation into crystal and mineral phases.

Anti-Mineralization Networks

Several factors, either systemic or local, can antagonize the aberrant mineralization of connective tissues, and several studies have noted direct association of these factors, such as fetuin-A, MGP, Ank, and alkaline phosphatase activity, with mineralization processes.⁵⁹ Fetuin-A, also known as α_2 -Heremans-Schmid glycoprotein, is a systemic inhibitor of mineralization. The critical role of fetuin-A as an anti-mineralization factor is attested by development of severe calcification in various organs of *Ahsg*^{-/-} mice, in which the gene encoding fetuin-A has been ablated.⁶⁰ This mineralization phenotype is not associated with changes in calcium and phosphate homeostasis, but results from decreased inhibitory activity of fetuin-A in serum of these animals. Ultrastructural and biophysical data have demonstrated that fetuin-A inhibits mineralization by incorporating into soluble colloidal spheres with calcium and phosphate. These

soluble calciprotein particles ordinarily become progressively more crystalline and insoluble in a time- and temperature-dependent manner, but the presence of fetuin-A facilitates solubilization of these particles, thus providing a mechanistic framework to remove insoluble calcium precipitates and transport them away from the site of mineralization.⁶¹

Another protein intimately linked to the regulation of tissue mineralization is MGP, which is expressed abundantly in vascular smooth muscle cells. Mice lacking MGP die within a few months of age because of mineralization of elastic fibers and rupture of elastic arteries, such as aorta.⁶² The presence of MGP has been demonstrated in association with elastic laminae in human arterial blood vessels, but such colocalization is lost in areas of vascular calcification, and MGP is found primarily in the extracellular matrix at the borders of vascular mineralization. These data support the notion of close association between MGP and the mineralization process and the role of the biologically inactive, undercarboxylated form of MGP in vascular calcification in vitamin K deficiency.⁶³

Collectively, the pathological processes leading to connective tissue mineralization in heritable disorders have both systemic and local regulatory factors, and the precise balance of promineralization and anti-mineralization factors, when superimposed on the individual's genetic background, determines the phenotypic features and severity of ectopic mineralization.

Pharmacological Perspective

There is no effective treatment for the systemic manifestations of ectopic mineralization disorders, such as PXE and GACI. In case of PXE, intravitreal injection of vascular endothelial cell growth factor antagonists prevents the neovascularization in the eyes, and significant improvement in the visual acuity has been reported.²³ However, the systemic mineralization in PXE progresses with advancing age, and the manifestations in the skin and the cardiovascular system become more evident. In case of GACI, some success has been reported by use of bisphosphonates, nonhydrolyzable pyrophosphate analogues, with carbon substituting for oxygen and two R groups that substitute for phosphate.⁶⁴ These drugs have been used to target bone, where they have anti-osteoclastic biological activity, in addition to physical inhibition of the formation of hydroxyapatite,

mimicking that of PP_i. The bisphosphonates were originally introduced as agents to treat Paget's disease and osteoporosis, disorders in which the osteoclasts are overly active. The first bisphosphonates to be introduced were weak anti-osteoclastic agents requiring their use in high doses, which resulted in an untoward adverse effect of osteomalacia, due to inhibition of bone mineralization. However, newer and more potent anti-osteoclast agents are used in lower doses, which reduce the inhibitory effects on bone mineralization. In GACI or other mineralization disorders, such as PXE, one does primarily want to inhibit mineralization with minimal effects on osteoclasts. Consequently, careful selection of the bisphosphonates for treatment of these disorders is required to maximize the inhibition of mineralization and minimize the adverse effects due to anti-osteoclastic activity.

Another approach to prevent systemic mineralization (ie, supplementation of the diet with magnesium) has been recently suggested to be potentially useful for treatment of patients with PXE. Early animal experiments feeding *Abcc6*^{-/-} mice with a diet fortified with magnesium up to levels that are five times higher than in the standard rodent diet completely prevented the connective tissue mineralization in these mice.⁶⁵ Conversely, reduction of the magnesium concentration to approximately 20% of that in control diet accelerated the mineralization process in the *Abcc6*^{-/-} mice.^{55,66} The mechanisms of inhibition of mineralization in this mouse model relate to formation of mineral complexes in which magnesium replaces calcium, and the excess calcium is excreted in the urine. Because magnesium phosphate complexes are more soluble under physiological conditions than those consisting of calcium phosphate, less mineralization occurs, potentially providing a useful treatment modality for patients with ectopic mineralization disorders. Based on these preclinical murine studies, a double-blinded clinical trial of patients with an experimental diet in which the magnesium content has been approximately doubled is under way (<http://clinicaltrials.gov/show/NCT01525875>, last verified November 2012). Because the progression of clinical signs and symptoms of PXE is slowly progressing and variable, and no biomarker is available to gauge the progress of mineralization, this study will be extended to 2 years to accurately document the efficacy of magnesium as an anti-mineralization agent. Because the mouse studies indicated that magnesium is effective in preventing the mineralization process, but not in reversing the existing mineral deposits, such dietary manipulation, if shown by the clinical trials to be effective, should be instituted as soon as the diagnosis has been established.

Acknowledgments

We thank Carol Kelly for her assistance with manuscript preparation, David Chou for preparation of Figure 1, and Qiujie Jiang and Haitao Guo (Jefferson Medical College), Eli Sprecher (Tel Aviv University), John P. Sundberg (The Jackson Laboratory), Annerose Berndt (University of

Pittsburgh School of Medicine), András Váradi (Hungarian Academy of Sciences), Michael A. Levine, Matthew A. Deardorff (Children's Hospital of Philadelphia), and Leon Schurgers (University of Maastricht) for their contributions to the original studies reviewed herein.

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